BioCurate

Fact Sheet

Small molecule tool compound validation – BioCurate's perspective

The aim of this document is to help investigators understand what constitutes a "validated small molecule tool compound" from an industry perspective.

"A tool compound is simply a reagent—a selective small-molecule modulator of a protein's function that allows the user to ask mechanistic and phenotypic questions about its molecular target in biochemical, cell-based or animal studies." Nature Chemical Biology, **2015**, *11*, 536

Like any reagent used in experimentation, small molecule tool compounds (chemical reagents) must undergo quality control (QC) prior to use. Tool compounds are only useful if they are potent, of known selectivity, and have a proven mechanism-of-action (MOA).

A small molecule tool compound can only be used to support Target Validation if it meets the following criteria*:

- Has sufficient **potency** to allow the experimental hypothesis to be tested. Potency must be determined by two orthogonal methods (e.g. biochemical assay and SPR)
- Has **selectivity** for the target of interest (e.g. screening against all closely related target family members and in broad pharmacology panels such as those available through Eurofins, DiscoveRx, CEREP). Off-target screening must be carried out at a relevant concentration to that used in the Target Validation experiments.
- Demonstrates evidence of **target engagement in cells** (e.g. CETSA)
- Is used in Target Validation experiments at an **appropriate concentration relative to its IC_{50}** (or EC_{50}) at the target of interest
- Shows evidence of **downstream biochemical effect** from modulation of its target
- Has an **inactive control compound** (a structurally related compound inactive at the target), and
- The compound **batch** to be used is checked for **purity and structural integrity.**

Quality tool compounds require significant resource and skill to generate and hence it's important to be aware that many of the tool compounds reported in the literature are not real (validated) – they may not be selective for the target or may not even engage (bind to) the claimed target.

When evaluating whether or not a literature tool compound is validated (or even real) one should be sceptical if any of the following features are present:

- No orthogonal confirmation of binding
- Only proliferation assay data
- No SAR or SAR appears lipophilicity driven
- Known PAINS functionality
- Frequent hitter structures, and/or
- No, or limited, selectivity data reported.

There are online resources available that list validated small molecule tool compounds:

- Chemicalprobes.org: http://www.chemicalprobes.org/
- The Structural Genomics Consortium: https://www.thesgc.org/

*Adapted from Nature *Chemical Biology*, 2015, *11*, 536

For further information on this, or any other topic related to the drug discovery and translation process, please email the BioCurate team on info@biocurate.com